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In vitro Evaluation of Fungicides and Biocontrol Agents Against *Damping Off Disease Caused by Sclerotium rolfsii* on Tomato

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ABSTRACT

An experiment was conducted with twenty-four fungal biocontrol agents and twelve bacterial biocontrol agents were screened for their efficacy against phytopathogenic fungi Sclerotium rolfsii through dual culture technique. The T. harzianum -1 and P. f-3 were found effective in inhibition of mycelium (95.1, 60.80) against Sclerotium rolfsii under in vitro conditions. Ten fungicides were tested for their efficacy against Sclerotium rolfsii tthrough poisoned food technique and the ten fungicides Mancozeb, Tebuconazole + Trifloxystrobin, Metiram + Pyraclostrobin, Cymoxanil + Mancozeb, Propiconazole and Captan + Hexaconazole were record 100 per cent at recommended and half the recommended dosage under in vitro condition.

Key words: *Biocontrol agents, T. harzianum, Pseudomonas fluorescence, Fungicides, Sclerotium rolfsii, Tomato.*

INTRODUCTION

Tomato (*Lycopersicon esculentum*. Mill) is one of the important vegetable crop in India and world. It is considered as 'Protective food' because of its nutritive value and year round production throughout the country. Soil borne fungal pathogens such as *Pythium* spp., *Rhizoctonia solani* and *Sclerotium rolfsii* infects the tomato crop causing damping off disease and is becoming a potential threat to its cultivation. *Sclerotium rolfsii*, a member of class Agaricomycetes is one of the important soil-borne plant pathogen and it causes great loss in agriculture production. This fungus like organism is an unspecialized parasite that has a wide host range. Young tissues and plants are infected and affected much more severely by this pathogen. It causes collar rot disease in several plants including tomato; it affects the plant both in pre and post emergence stage in nursery beds and pots. Even though this pathogen can be controlled by some fungicides, nowadays researchers are more interested on biological control agents and their antifungal metabolites due to the notification of resistance development in the pathogen. Biological control is an alternative approach to the chemical fungicides and it may be a safe, effective and ecofriendly method for plant disease management. Collar rot is an important disease of tomato, causing significant losses in nurseries where young susceptible transplants are produced.

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Therefore, in the present study an attempt was made to test the feasibility of bio-control and fungicides ability of twenty-four different *Trichoderma* spp., twelve different *Bacillus* spp. and different *Pseudomonas* spp and different fungicides against tomato damping off causing pathogen *Sclerotium rolfsii*.

MATERIAL AND METHODS:

Isolation and Identification

The test pathogen *Sclerotium rolfsii* was isolated from the disease tomato samples and collected from the farmer's field. Identification of *Sclerotium rolfsii* fungus produced white, dense radiating mycelial growth on PDA. In early stages of growth, the mycelium was silky white and became dull in appearance and colonies grew radially. Sclerotial initials were observed from 6th day on wards. At the initial stage, the bodies were white in colour later they turned buff brown colour to chocolate brown at maturity. Matured sclerotia were spherical to ellipsoidal. On the basis of these characters the fungus was identified as *Sclerotium rolfsii*[4,9].

The pathogenicity of *Sclerotium rolfsii* was tested on tomato (cv Arka vikas) by adopting soil inoculation method. Profuse white mycelial growth was found on the soil surface after 24 h after inoculation. White cottony growth at collar region and root zone was observed in wilted plants. Numerous round brown and mustard seed like sclerotia were seen on soil surface and root region of the infected plants and 9 days after inoculation. The pathogen was re-isolated from affected plant tissue and fungus obtained resembled the original isolate in all respects and based on the morphological characteristics it was confirmed as *Sclerotium rolfsii.*

Isolation of fungal and bacterial bio control agents

About 24 isolates of fungal biocontrol agents and 12 bacterial bio control agents were isolated from the rhizosphere samples of tomato collected in Ranga Reddy district. Particularly *Trichoderma* spp, *Pseudomonas* spp and *Bacillus* spp were isolated. Further morphological characteristics of these isolates were studied and identified based on the key characteristics provided by Rifai[12].

Identification of fungal and bacterial Bio control agents

For isolation of *Trichoderma* strains, a serial dilution technique was followed. For this purpose one millilitre of each solution was pipetted onto a Rose Bengal Agar (RBA) plate and incubated at 28°C for 1 week. The culture plates were examined daily and each colony that appeared was considered to be one colony forming unit (cfu). After enumeration of cfu, individual colonies were isolated from the same plates and each uncommon colony was reisolated onto a fresh Potato Dextrose Agar (PDA) plate. Distinct morphological characteristics were observed for identification, and the plates were stored at 4°C. Two techniques, visual observation on petri dishes and micro-morphological studies in slide culture, were adopted for identification of *Trichoderma* species. For visual observation, the isolates were grown on PDA agar for 3-5 days. The mode of mycelia growth, colour, odour and changes of medium colour for each isolate were examined every day. For micromorphological studies, a slide culture technique was used. Examination of the shape, size, arrangement and development of conidiophores, their branching pattern, shape, size, angle to main axis, phialide numbers and conidial shape and colour. Species identification was based on the morphological and taxonomic keys provided by Bisset. Rhizosphere soil samples were screened for *Pseudomonas* spp and *Bacillus* spp. using dilution method with King's B Agar as semi selective medium. *Pseudomonas* spp and *Bacillus* isolates were estimated by morphological and physiological characteristics based on Bergeys' Manual of Systematic Bacteriology.

Screening of fungal and bacterial bio control agents

The rhizosphere microorganisms isolated from tomato plants were screened for their antagonistic activity against the test pathogen *Sclerotium rolfsii* by following dual culture technique[2]. About twenty isolates of

Trichoderma spp and twelve native *Pseudomonas* spp and *Bacillus* spp. isolates were obtained from rhizosphere soil and maintained by periodical sub-culturing.

Testing antagonistic activity of fungal bio-control agent

The test antagonists *Trichoderma* spp. were tested against test pathogen *Sclerotium rolfsii* and they were grown on the same plate to test the antagonistic activity. About 15 to 20 ml of melted and cooled PDA medium was poured in to Petri plates and allowed to solidify. Fungal disc of the antagonist of was placed at one end of media on Petri plate. A 9 mm test pathogen PDA culture disc was placed at the opposite end. Four replications along with suitable control were maintained. The plates were incubated in an inverted position at room temperature (25 ± 2^0 C) till the mycelial growth in the control plates covered the entire plate. The radial growth of the pathogen was measured and the percentage inhibition was calculated by adopting following formula.

$$R = \frac{CD - TD}{CD} \times 100$$

Where,

R = Per cent growth reduction of test pathogen

CD = Radial growth of test pathogen in check (mm)

TD = Radial growth of test pathogen in treatment (mm)

Testing antagonistic activity of bacterial bio-control agent

The antagonistic activity of native bacterial isolates spp. was tested against the test pathogen *Sclerotium rolfsii*, by following dual culture technique. A gentle superficial streak was made at four ends of the Petri plate on PDA medium by means of a sterilized inoculation needle. A nine mm PDA culture disc of the pathogen was placed in middle of Petri plate. Three replications along with suitable control were maintained. The plates were incubated in an inverted position at room temperature (25 ± 2^0 C) till the mycelial growth in the control plates covered the entire plate. The radial growth of the pathogen was measured and the percentage inhibition was calculated by adopting following formula.

$$R = \frac{CD - TD}{CD} \times 100$$

Where,

R = Per cent growth reduction of test pathogen

CD = Radial growth of test pathogen in check (mm)

TD = Radial growth of test pathogen in treatment (mm)

Evaluation of different Fungicides Against *Sclerotium rolfsii*:

Ten fungicides were evaluated against *Sclerotium rolfsii*, by poisoned food technique[11] at two concentrations *i.e.* at recommended dose and half the recommended dose. The required quantities of fungicides were weighed and mixed in the carrot agar medium by thorough shaking for uniform mixing of the fungicide before pouring into Petri dishes so as to get the desired concentration of active ingredient of each fungicide separately *i.e.* recommended and half the recommended doses. Twenty ml of amended medium was poured in 90 mm sterilized Petri dishes and allowed to solidify. Mycelial discs of 5 mm diameter from 7-day old culture was inoculated at the centre of the Petri plate and then incubated at 18±2^0C for 7 days. Control was maintained without fungicide. Three replications were maintained for each treatment. Per cent inhibition of mycelial growth was calculated using the formula[14].

$$I = (C-T/C) \times 100$$

Where

I = Per cent inhibition of mycelial growth

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

Table 1: List of fungicides tested against *Sclerotium rolfsii* by poisoned food technique under *in vitro* conditions

Common name	Trade name	Recommended concentration	Half recommended concentration
Mancozeb	Dithane M-45	0.3 g l^{-1}	0.15 g l^{-1}
Pyraclostrobin		0.1 g l^{-1}	0.05 g l^{-1}
Tebuconazole + Trifloxystrobin	Nativo	0.4 g l^{-1}	0.2 g l^{-1}
Copper Oxy Chloride	COC	0.3 g l^{-1}	0.15 g l^{-1}
Metiram + Pyraclostrobin	Cabriotop	0.2 g l^{-1}	0.1 g l^{-1}
Cymoxanil + Mancozeb	Curzate	0.08 g l^{-1}	0.04 g l^{-1}
Propiconazole	Tilt	0.1 ml l^{-1}	0.05 ml l^{-1}
Captan + Hexaconazole	Taqat	0.1 g l^{-1}	0.05 g l^{-1}
Thiram	Thiride	0.3 g l^{-1}	0.15 g l^{-1}
Carbendazim	Bavistin	0.15 g l^{-1}	0.075 g l^{-1}

RESULTS AND DISCUSSION

Screening of native fungal bio-control agents on the growth of *Sclerotium rolfsii*

An attempt was made to evaluate the native isolates of *Trichoderma* spp against *Sclerotium rolfsii* causing collar rot / damping off of tomato. The results presented in Table-2 and Fig.1 showed that all the *Trichoderma* isolates were found to be significantly superior in inhibiting *S. rolfsii*. Among all the isolates the isolate – *T. harzianum* -1 showed maximum (95.1) per cent inhibition of *Sclerotium rolfsii* followed by *T. harzianum* -2 (95.0) and *T. viride*-10 (94.4). Minimum per cent inhibition was recorded by *T. harzianum*-9 (57.23). It was observed that most of the *Trichoderma* isolates recorded 90 per cent inhibition of *Sclerotium rolfsii*.\The antagonistic ability of different isolates of *Trichoderma* spp. including *T. harzianum* was reported by Das *et al*[3]., Rajani[13], Karthikeyan *et al*[5].

Kulkarni[6] evaluated *Trichoderma* spp *viz*., *T. harzianum*, *T. viride*, *T. virens*, *T. koningii* against *S. rolfsii* causing potato wilt and *T. harzianum* was found most effective in inhibiting the *S. rolfsii*. The finding of Kulkarni[6] provides a positive support to our present findings that most of the antagonists inhibited the growth of *S. rolfsii* to variable degree.

Madhavi and Battiprolu[7] tested five bio-agents against *S. rolfsii* and found that the isolate *T. harzianum* was found to be significantly effective against *S. rolfsii* under *in vitro* and *in vivo* condition.

Screening of native bacterial bio-control agents on the growth of *Sclerotium rolfsii*

Twelve native bacterial isolates were tested for their antagonism against *Sclerotium rolfsii* and the results are presented in Table-3 and Fig.2. All the isolates were inhibitory towards the radial growth of the test pathogen *S. rolfsii*. *P. fluorescence*-3 was found to be effective in inhibiting the radial growth of pathogen with a maximum inhibition of 60.80 per cent followed by the *B. subtilis* -6 (59.6), *B. subtilis* -2 (57.4) and *B. subtilis* -4 (57.3). However, significant difference was not observed between these isolates in inhibiting *S. rolfsii*. The *B. subtilis* -3 recoded least inhibition (42.13) of mycelial growth of test pathogen. However, the bacterial isolates *P. f*-6, *B. subtilis* -4, 2 and 6, *Bacillus subtilis* isolates -1, 5 and *P. f* -4, *B. subtilis* isolate -3, *P. f* isolates-1and 2 were on par with each other in inhibiting *Sclerotium rolfsii*.

Arunasri[1] made similar observation with various bacterial isolates where *Pseudomonas* spp was found effective in inhibiting the mycelial growth and sclerotial production of *S. rolfsii* with 43.10 and 71.0 per cent respectively. In the present study, *Pseudomonas fluorescence*-3 was found to be superior in inhibiting the mycelial growth under *in vitro* conditions when compared the control. Similarly, Karthikeyan *et al*[5]., and Kulkarni[6] reported that *P. fluorescence* and *Trichoderma* spp inhibited *S. rolfsii* causing stem rot of ground nut.

In vitro* evaluation of fungicides against *Scleortium rolfsii

The data presented in Table-4, Plate 10 and Fig.3 indicated that there was significant difference between fungicides in inhibiting the test pathogen *Sclerotium rolfsii.* Mancozeb, Tebuconazole + Trifloxystrobin, Metiram + Pyraclostrobin, Cymoxanil + Mancozeb, Propiconazole, Captan + Hexaconazole recorded maximum (100.0) inhibition of *S. rolfsii* at recommended dosage whereas fungicides, Pyraclostrobin, Thiram, Carbendazim and COC recorded an inhibition of 96.97, 90.13, 69.40 and 60.83 respectively.

The fungicides Mancozeb, Tebuconazole + Trifloxystrobin, Metiram + Pyraclostrobin, Cymoxanil + Mancozeb, Propiconazole, Captan + Hexaconazole recorded maximum (100.0) per cent of inhibition of radial growth of *S. rolfsii* at half recommended concentration. The fungicides Pyraclostrobin, Thiram and Carbendazim showed 92.93, 84.77 and 62.55 per cent inhibition of test fungus *S. rolfsii* while least inhibition of 58.4 per cent was observed by COC. The fungicides, Mancozeb, Tebuconazole + Trifloxystrobin, Metiram + Pyraclostrobin, Cymoxanil + Mancozeb, Propiconazole, Captan + Hexaconazole found to be effective over other fungicides at both concentrations tested *i.e.* recommended and at half the recommended dosage.

Out of 10 fungicides tested, five fungicides *viz*., Mancozeb, Tebuconazole + Trifloxystrobin, Propiconazole, Cymoxanil + Mancozeb, Captan + Hexaconazole were found to be effective in reduction of mycelium growth of *S. rolfsii* at recommended and half recommended concentration. The efficacy of Propiconazole inhibition of fungal growth was also reported by several others. Mukherjee and Tripathi[10] reported Propiconazole as best fungicide against *S. rolfsii.* Similarly, Manu *et al*[8]., reported Tebuconazole +Trifloxystrobin and Mancozeb as best fungicide to control *S. rolfsii* causing foot rot of ragi.

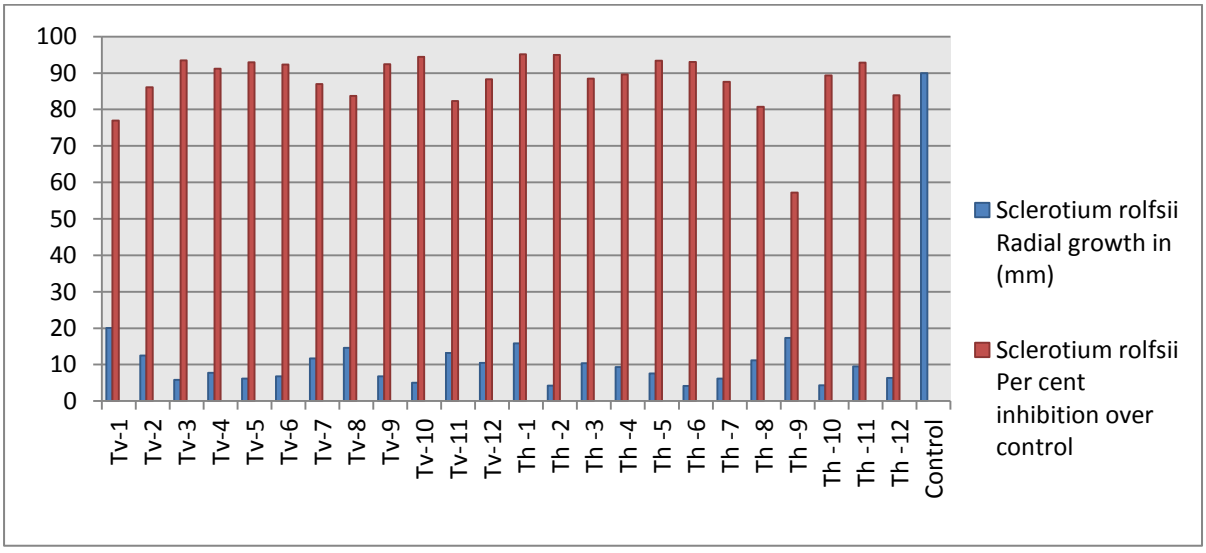

Fig. 1: Antagonistic activity of native *Trichoderma* isolates against *Sclerotium rolfsii*. (T.v Means - *Trichoderma viride*, Th- *Trichoderma harzianum)*

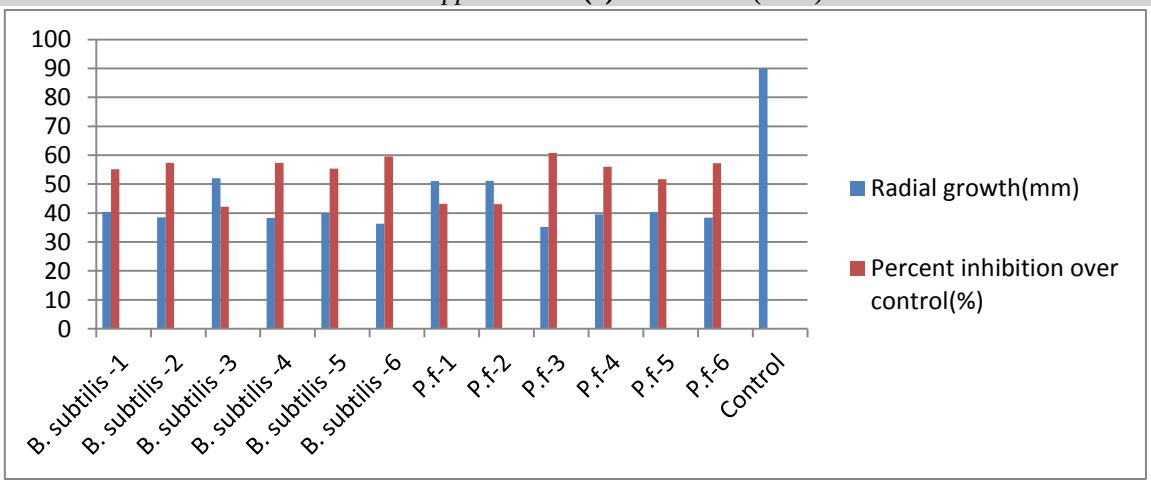

Fig. 2: Effect of native isolates of bacterial bio-agents on radial growth of *Sclerotium rolfsii* (B.S- Means *Bacillus subtilis*) (P.f-*Pseudomonas fluorescence)* isolates

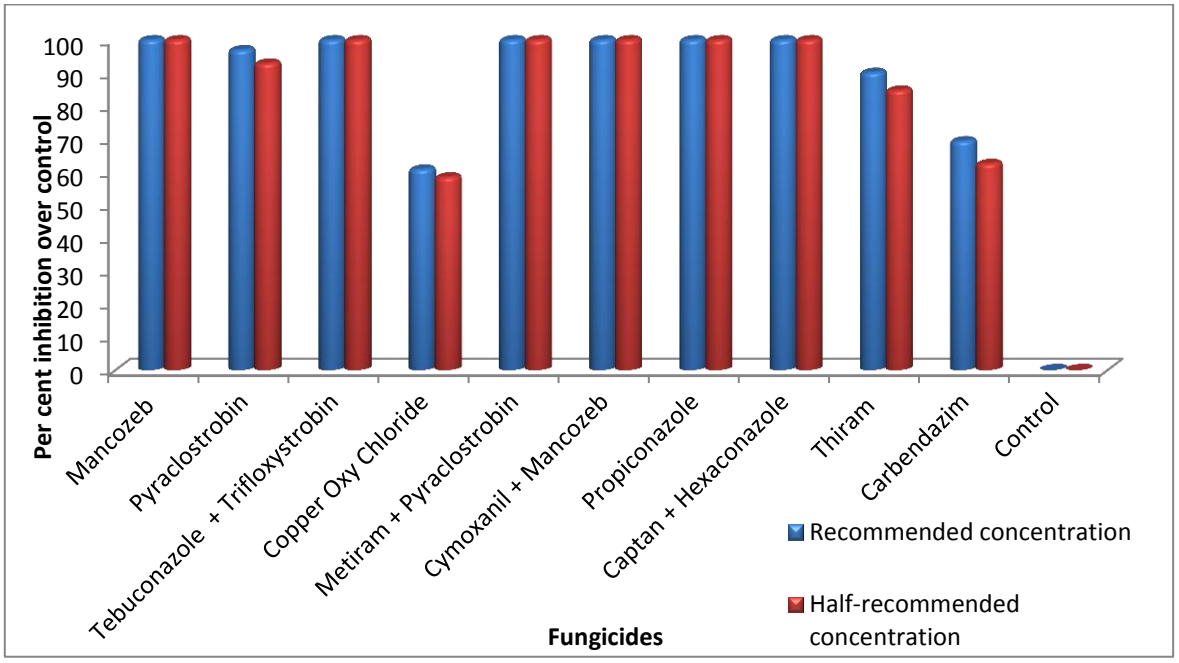

Fig. 3: *In vitro* evaluation of Recommended and Half-recommended dosages of fungicides on radial growth *Sclerotium rolfsii*

Table 2.A) Antagonistic activity of native *Trichoderma* isolates against *Sclerotium rolfsii*

Treatment	*Radial growth in (mm)	*Per cent inhibition over control (%)
Trichoderma viride-1	20.6	77.00 (61.43)
Trichoderma viride-2	12.5	86.10 (68.09)
Trichoderma viride-3	5.80	93.46 (75.95)
Trichoderma viride-4	7.80	91.23 (72.82)
Trichoderma viride-5	6.20	92.93 (74.58)
Trichoderma viride-6	6.80	92.36 (74.10)
Trichoderma viride -7	11.70	86.96 (68.90)
Trichoderma viride-8	14.62	83.73 (66.27)
Trichoderma viride -9	6.76	92.43 (74.42)
Trichoderma viride-10	5.00	94.40 (77.08)
Trichoderma viride -11	13.2	82.36 (65.16)
Trichoderma viride -12	10.52	88.26 (70.01)
Trichoderma harzianum -1	15.82	95.1 (77.27)
Trichoderma harzianum -2	4.22	95 (77.09)
Trichoderma harzianum -3	10.36	88.43 (70.36)
Trichoderma harzianum -4	9.30	89.63 (71.27)
Trichoderma harzianum -5	7.62	93.36 (75.08)
Trichoderma harzianum -6	4.12	93.06 (74.91)
Trichoderma harzianum -7	6.22	87.56 (69.35)
Trichoderma harzianum -8	11.18	80.7 (63.92)
Trichoderma harzianum -9	17.32	57.23 (77.49)
Trichoderma harzianum -10	4.32	89.36 (71.08)
Trichoderma harzianum -11	9.52	92.86 (74.79)
Trichoderma harzianum -12	6.36	83.86 (66.00)
Control	90.00	
CD at 5% SE(d) SE(m)	5.208 2.582 1.826	

***** *Mean of three replications*

Figures in parentheses are angular transformed values

Table 2.B) Antagonistic activity of native isolates of bacterial bio-agents against *Sclerotium rolfsii*

Treatment	*Radial growth in (mm)	*Per cent inhibition over control (%)
Bacillus subtilis -1	40.36	55.10 (47.91)
Bacillus subtilis -2	38.50	57.40 (49.26)
Bacillus subtilis -3	52.06	42.13 (40.45)
Bacillus subtilis -4	38.36	57.30 (49.19)
Bacillus subtilis -5	40.02	55.30 (48.02)
Bacillus subtilis -6	36.30	59.60 (50.55)
Pseudomonas fluorescence-1	51.02	43.23 (41.09)
Pseudomonas fluorescence-2	51.16	43.10 (41.01)
Pseudomonas fluorescence-3	35.22	60.80 (51.21)
Pseudomonas fluorescence-4	39.52	56.03 (48.44)
Pseudomonas fluorescence-5	40.34	51.70 (45.95)
Pseudomonas fluorescence-6	38.42	57.23 (49.14)
Control	90.00	-
CD at 5% SE(d) SE(m)	3.10 1.49 1.05	

***** *Mean of three replications*

Figures in parentheses are angular transformed values

Table-3) *In vitro* evaluation of fungicides on radial growth of *Sclerotium rolfsii*

S.No.	Fungicides	Recommended concentration		Half recommended concentration	
		*Radial growth (mm)	*Per cent inhibition over control (%)	*Radial growth (mm)	*Per cent inhibition over control (%)
1	Mancozeb	0	100.0 (90.00)	0	100.0 (90.00)
2	Pyraclostrobin	2.6	96.97 (80.24)	6.3	92.93 (74.87)
3	Tebuconazole + Trifloxystrobin	0	100.0 (90.00)	0	100.0 (90.00)
4	Copper oxy chloride	35.2	60.83 (51.23)	37.3	58.47 (49.85)
5	Metiram + Pyraclostrobin	0	100.0 (90.00)	0	100.0 (90.00)
6	Cymoxanil + Mancozeb	0	100.0 (90.00)	0	100.0 (90.00)
7	Propiconazole	0	100.0 (90.00)	0	100.0 (90.00)
8	Captan + Hexaconazole	0	100.0 (90.00)	0	100.0 (90.00)
9	Thiram	8.8	90.13 (72.62)	13.6	84.77 (67.01)
10	Carbendazim	27.5	69.40 (56.39)	33.8	62.55 (52.25)
11	Control	90	0.00	90	0.00
	CD SE(d) SE(m)		4.477 2.131 1.507		2.380 1.133 0.801

***** Mean of three replications

Figures in parentheses are angular transformed value

Plate-10: *In vitro* **evaluation of fungicides on the radial growth of *Sclerotium rolfsii***

CONCLUSION

Among the 24 fungal and 12 bacterial biocontrol agents were tested for their antagonistic activity against *S. rolfsii* and the *T. harzianum* -1 (95.1) and *P. f*- 3 (60.80) per cent recorded maximum inhibition of mycelium against *S. rolfsii*.

Among ten fungicides tested Mancozeb, Tebuconazole + Trifloxystrobin, Metiram + Pyraclostrobin, Cymoxanil + Mancozeb, Propiconazole and Captan + Hexaconazole against *S. rolfsii*, showed 100.0 per cent inhibition (100.0 per cent) at recommended and half the recommended dosage under *in vitro* condition.

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